

ABSTRACT

High Density Gridded Array Producing Using a Second Generation Spotting System

We describe recent enhancements to our high density filter production system. Genome researchers at LLNL continue to make use of high density gridded arrays of transformed bacteria or DNA to support sequencing and gene finding efforts. We recently purchased a robotic positioning system to supplement our Hewlett-Packard ORCA robot. We have used the ORCA for the past three years to produce high density gridded arrays at 6x6x96 density, each containing 3,456 spots. The new system will allow us to produce high density arrays containing up to 98,000 spots on an 8-cm x 12-cm substrate. We have also made some process improvements which streamline filter production.

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As part of this development effort we are investigating the use of fluorescent probes and associated detection systems. We have also made some improvements to the procedure for growing colonies on nylon and for lysis and fixing of the cells. In particular, we have focused on modifications that will increase throughput or facilitate automation. We are now growing colonies on media saturated wicking paper instead of LB agarose. We have investigated a chemical lysis protocol and designed and built a fixture that allows more efficient batch processing of colony filters. We have also developed a 384-pin tool using pins from the semiconductor industry which is more accurate than our previous 384-pin tool. In addition, the majority of the components of the new tool are commercially available.

All of our cosmid libraries have been rearranged into 384-well plates and we are currently producing arrays at a 6x6 density using the 384-pin tool. The new spotting robot has a work envelope of 0.25m x 1m x 2m and allows us to produce up to 186 8-cm x 12-cm filters at a time.

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